REPORT TO THE MEDICAL RESEARCH COUNCIL

On the work of the Division of Molecular Genetics, now the Division of Cell Biology, from 1961 - 1971.

by

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INTRODUCTION

When the laboratory was set up in 1961 molecular biology was in the latter part of its classical period. This started with the discovery of the structure of DNA in 1953 and lasted till the elucidation of the major part of the genetic code in 1965.

The basic ideas which powered this revolution are now too well-known to require detailed description. Nucleic acid is the genetic material. It replicates by complementary base-pairing. The amino acid sequence of every protein is specified by the base sequence of a piece of nucleic acid, each amino acid being coded by a triplet of bases. The usual sequence of information transfer is thus from gene to polypeptide chain via messenger RNA as an intermediate. That is

$$\text{DNA} \rightarrow \text{mRNA} \rightarrow \text{protein.}$$

Protein synthesis usually takes place in the cytoplasm, using an elaborate mechanism consisting of activating enzymes, transfer RNA, ribosomes and various factors. When a polypeptide chain has been produced it folds itself up into its characteristic three-dimensional structure. A fuller description is given in Watson's text book.[a]

Just before we moved, our two major contributions had been the discovery (3) of messenger RNA (predicted by Jacob and Monod[b] and also discovered by Francois Gros and his colleagues[c] in Watson's laboratory) and of phase-shift mutations produced by acridines (2). The latter led to the first evidence that the genetic code was in fact a triplet code (5).

After we had settled into the new laboratory, we consolidated the genetic

work on the phase-shift mutants (65, 77). Much further genetic work went towards establishing the chain termination triplets of the genetic code, and (in parallel with Yanofsky [d]) it was shown that the gene and the protein for which it codes are collinear. Extensive experimental projects were started on transfer RNA, especially the special methionine tRNA used in chain initiation. We studied the mechanism of suppression, the production and properties of many tRNA mutants, the crystallisation of tRNA and, more recently, the base-sequence of a tRNA precursor molecule. Parallel studies dealt with various aspects of protein synthesis.

Much of this work has been done in informal collaboration with Sanger's Division, partly because the sequencing methods he developed were so useful to us, and partly because of the influence and personality of Dr. Marcker.

All this work, some of which is still continuing, was necessary because, although the basic scheme of molecular biology had been established in outline, many important details needed to be filled in. Even to this day the main mechanism of DNA replication in the cell has not been established. A typical problem which could have occupied us usefully for ten years was the exact structure and function of ribosomes. Indeed, in many ways, the laboratory as a whole was ideally suited to the problem, since we are highly expert in protein and nucleic acid sequencing, the determination of tertiary structures and have much experience in the complexities of protein synthesis.

In spite of this, we decided in our Division to make our main research efforts in other directions. We reasoned that many laboratories, especially in the United States, would certainly tackle the type of problem mentioned above,

that the field was already overcrowded and that we could more usefully attempt to pioneer areas of biology which were, by comparison, relatively backward .

It was not an easy matter to choose which areas to study, since the totality of biology is so vast. Most of the work in classical molecular biology had been done on micro-organisms (mainly for technical reasons) and it was known that many of the results also applied at least to some extent to higher organisms. However, higher organisms have problems not found in single-celled systems. Many different cell types exist which interact in complex ways to produce tissues and organs. Thus it was clear that we should embark on some aspects of the biology of the higher eukaryotes.

Our general principle was to look for problems which were of very wide biological significance and yet at first sight appeared to defy explanation. It is exactly such problems which, if solved, produce major revolutions in our understanding of Nature. Moreover, the fact that they are universal makes one hope that there may be an underlying simplicity. Notice that these were the features which characterised molecular biology in the '40s. The nature of reproduction and the border-line between the living and the non-living had, at that period, just this fascination and difficulty.

It appeared to us that two broad areas had the characteristics we were looking for. The first was embryology (or developmental biology as it is now called). The problem of how to build a hand, with the correct blood supply, bones and muscle, highly innervated and covered with epidermis is a deep mystery, as can be seen by asking the simple question, "How many genes are needed to programme for it?"

The second area was the higher nervous system, and especially its

integrated behaviour.

Naturally our aim was not to consider complete solutions to these problems but to try to bring them to the stage now reached by classical molecular biology, where the main framework of explanation has been established and an army of professionals is deepening the picture and filling in the details. If this end could be achieved in either of these fields, it would have, in the long run, a profound impact on medical problems and our understanding of man's place in Nature.

In the upshot our main effort has been directed to developmental biology. So far our studies of the nervous system have been more on its development than on its integrated behaviour. Obviously we would not hope to cover more than a fraction of the possible approaches. Much of the work of the last five years, and especially of the last two years (since we moved into the new extension to the laboratory) has been to explore several possible methods of attack in a preliminary way.

Our approach was not to take up some well-studied experimental system and apply modern methods to it. This was unnecessary because those workers already in the field were doing this. Instead, we attempted a theoretical analysis of the problems involved. In particular, we first tried to split them up into well-defined sub-problems and then looked for suitable experimental systems for each sub-problem. This explains the investment in nematodes (because of their relatively small number of cells and their favourable genetics), the choice of the insect cuticle and a blue-green alga to study positional information, our interest in fibrillar elements as the major tools used by the cell for movement and the determination of cell shape, and the

work on the location of proteins in membranes.

In the future the main emphasis of the Division will be on the new problems, but we will continue work on the classical molecular biology of micro-organisms since it is still producing interesting results. However, in the long run, it may be that the entire Division will be working on higher organisms.

The next section of this report has three sub-sections covering the work on micro-organisms. The first, on protein synthesis, has been deliberately made as brief as possible since much of the work was done some time ago. The second (nonsense mutants, chain termination and suppression) has been given in somewhat greater detail because collectively it was a major achievement of the Division. The third describes our work on transfer RNA.

The section following, entitled 'Cell Biology', is divided into five sub-sections covering much of the new work now in progress. Other lines of work in progress together with a brief reference to theoretical work and to work discontinued are listed in the section headed 'Miscellaneous'. Finally, we have added some general remarks about the future. At the end a list is provided of all the papers published during the last ten years.

2. MICROBIOLOGY

A. The mechanism of protein synthesis

We shall only deal very briefly with the early work in this area. Mark Bretscher, while still a research student, was the first person (in collaboration with Dr. Marianne Grunberg-Manago) to show that an artificial messenger RNA could be translated even though it contained no uracil (12). He also obtained suggestive evidence that an RNA polymer containing only U and A in a random order would produce polypeptide chain termination, but this was followed up only at a much later date (37).

Our work on protein synthesis was mainly done by Drs. Brian Clark, Mark Bretscher, Robin Monro and their collaborators. Much of it concerned chain initiation and sprang from Marcker and Sanger's discovery of the special initiator tRNA for formyl-methionine^{e}. This part of the work was mainly done in collaboration with Dr. Marcker in Sanger's Division.

It was discovered that there were two distinct transfer RNA molecules which accepted methionine (53, 54). One of these inserted methionine in the middle of polypeptide chains. The other was always used to initiate a polypeptide chain and, moreover, its methionine was normally formylated. In most cases the formyl group or the entire formyl-methionine was removed from the growing chain by a subsequent enzymatic step.

The base sequence of both these tRNAs from E. coli was worked out (89, 90, 93, 108, 109, 123). The triplets recognised by the special initiator tRNA were studied in a cell-free system in relation to the requirement for GTP and the various special protein factors (63, 64, 82). A by-product of the sequence work was a set of large fragments of the f-met-tRNA and it was

shown that a fragment containing the anticodon loop and its stem could bind specifically and recognize the AUG triplet (87, 116).

An important tool in this work was the use of the antibiotic puromycin which produces premature chain termination (31, 46, 52, 73). In parallel Dr. Robin Monro was studying peptide bond formation and the way it could be interfered with by various inhibitors (58, 74, 79). Among other things he was able to show that it was the larger sub-particle of the ribosome which catalyzed peptide bond formation (101, 115).

Further biochemical work in protein synthesis has been concentrated on the role of the specific protein factors in chain initiation. Dr. Phillip Rudland purified the initiator factor F2a and showed that it formed a specific complex with f-met-tRNA and GTP. This suggests that F2a acts as a special transfer factor for initiation, analogous to the Tu factor for chain propagation (117, 166). A special biological role for the initiator factors is suggested by experiments on ribosomes from T4 phage infected cells (129, 140). These ribosomes lose their capacity to recognize the initiation sequences of R17 and do not bind E. coli messenger RNA, while binding well to phage messenger. The difference lies in the factor fraction, and it is likely that part of the switch in protein synthesis after phage infection is due to an alteration in the specificity of initiation.

Much of the later work was on chain initiation of a natural messenger, the RNA of the bacteriophage f2. Dr. Harvey Lodish (95, 96, 97) showed that the three genes on this RNA were independently translated in an in vitro system and that the polarity effects of amber mutants of the coat cistron could be explained by a failure to expose the initiation sites of the synthetase gene.

More recently the knowledge gained about initiation has been turned to good account by using the formation of the initiation complex as a means of finding the base-sequence involved in that part of the message. The triplet AUG which is used for chain initiation is also the only one available for methionine. For this, if for no other reason, AUG must occur sometimes in the middle of RNA sequences coding for polypeptide chains. Thus the cell must in some way distinguish the special AUG used for chain initiation from all the others. It was surmised that this would depend mainly on the sequence in the immediate neighbourhood of the triplet.

Dr. Joan Steitz set out to do this by forming the initiation complex with the single-stranded RNA of bacteriophage R17 (which is closely related to f2). She then digested away all the RNA not protected and proceeded to obtain the base sequence of what remained, using once again the elegant methods developed by Sanger for RNA sequencing.

The results showed that there were three such sequences, one for each of the three proteins coded for by the virus (118, 119). Two of the sequences immediately before the AUG triplet were similar but not identical to each other. The third was somewhat different. The exact significance of these sequences is still not understood.

The same technique is now being applied to find initiation sequences on single-stranded DNA. This is not normally used by the cell as a messenger but Bretscher had shown that a special initiation complex could nevertheless be formed. In fact, in special circumstances, translation can be forced and he used this to show that single-stranded DNA can act as a message even if it is circular (81, 104, 106). Dr. Hugh Robertson is now collaborating with

Sanger in an attempt to find the initiation sequences on the single-stranded DNA phage fd. It is more difficult to sequence DNA than RNA but the preliminary results look promising.

B. Nonsense mutants, chain termination, suppression.

By 1961 genetical and biochemical experiments had revealed the general nature of the genetic code. Although further biochemical experiments were soon to lead to the assignment of triplets to the twenty different amino acids, the question of "nonsense" in the code was a confused one. A class of mutants existed which produced drastic effects on gene expression and which could often be suppressed by certain special genes elsewhere. These were loosely called nonsense mutants but nobody knew what the exact consequences of such mutations were, or anything about the mechanism of their suppression.

Our interest in this field grew partly out of the long search for an experimental system to solve collinearity. It occurred to us that a nonsense mutant might produce premature termination of the polypeptide chain and the release of a peptide fragment. For most proteins, the purification of such fragments would be a formidable problem but, in the favourable case of the head protein of phage T4, which represents more than half the protein synthesis of the phage-infected cell, it was thought that fragments could be identified without any purification. We were able to show that one class of nonsense mutants (called amber mutants) did in fact lead to chain termination and we used this result to prove that the genetic map is collinear with the polypeptide chain (27).

At the same time, more genetic work was done on amber mutants and their suppressors. It was clear that the strains used by other workers were

far from isogenic and so a canonical set of suppressor strains was derived. Another class of nonsense mutant, called ochre, was characterised and suppressors of these isolated and mapped (33, 45). Using the canonical set, we showed that in the case of the amber mutants of the head protein, chain termination occurred at the site of the mutation and that the suppressors competed with this process (48). The three suppressors su_I^+, su_{II}^+, su_{III}^+ were shown to insert serine, glutamine and tyrosine with efficiencies of 65%, 30% and 50% respectively (40).

We found that the effects of a nonsense mutation could be removed by placing it within a frame-shift (34). Because of this, we argued that the effects had to be expressed during protein synthesis. This suggested that such mutations produced one or other of the special triplets used for chain termination. By a combination of genetic experiments on the r_{II} gene of phage T4 and a study of the amino acid triplets related to the amber and ochre triplets we concluded that the latter were UAG and UAA respectively (35, 62, 78).

Later, we showed (66) that a third class of nonsense triplet is UGA and we isolated a strong suppressor for this triplet (75). Initially, we thought that a special transfer RNA might exist for normal chain termination and much work was done to search for it (83). However, the discovery that UGA is also nonsense made this unlikely on the basis of the wobble hypothesis (55) and it remained for others to show later that chain termination does not involve recognition of triplets by tRNA but by special proteins.

All our later work on this problem has been on the exact mechanism of

suppression. Theoretical considerations led us to the view that nonsense suppressors could arise by single base changes in the anticodons of amino acid tRNAs provided that there was more than one tRNA for that amino acid triplet and that suppression of natural chain termination was not too detrimental to the cell.

In principle, this could be shown by comparing the nucleotide sequences of the appropriate tRNAs but purification seemed to be a formidable problem, particularly because it was suspected that the suppressor tRNAs were present in only small amounts. This difficulty was overcome by placing the amber suppressor gene $\underline{su}^{+}_{III}$ (which suppresses by inserting tyrosine) onto a bacteriophage Ø80. Infection of cells with this phage produced dramatic enhancement of the synthesis of this particular RNA because of the multiplication of the phage genome. Using the sequence methods developed by Sanger, Dr. John Smith and his collaborators quickly showed that E. coli had two tRNAs for tyrosine, a major and minor, with different sequences, and that the minor one was produced by the suppressor gene (61, 94, 130, 137). In the $\underline{su}^{-}$ strain the anticodon contained the sequence -GUA-, consistent with the recognition by this tRNA of the two tyrosine triplets UAC and UAU. In the su^{+} strain an anticodon sequence of -CUA- was found which recognises the amber triplet UAG. Recently, using a special ochre suppressor variant of this tRNA we were able to show the change in its anticodon to UUA expected from the recognition of both UAA and UAG by this suppressor (143).

It soon became clear that this gene offered an excellent opportunity to study the relationship between structure and function of a tRNA since it

enabled one to obtain mutants of a dispensable tRNA. Mutants of this tRNA were isolated and their synthesis and sequences studied. This work is discussed in the next section.

In 1968 Dr. David Hirsh began work on the UGA suppressor (131, 154, 155). Genetic experiments suggested that this suppressor inserted tryptophan tRNA in the UGA su$^+$ strain. He proved that this was associated with a single base change in the tRNA but, remarkably, this was not in the anticodon (as might have been expected) but in the stem of the dihydrouracil loop. There is only one type of tryptophan tRNA in E. coli and all of the molecules are altered by the mutation. It appears that, in this case, the anticodon CCA can recognize the UGA triplet poorly, since UGA mutants are always slightly leaky. This anomalous recognition can be enhanced by a change elsewhere in the molecule which, at the same time, alters its physical properties so that it is more stable.

Dr. William McClain (135) discovered su$^+$ mutants of bacteriophage T4 capable of suppressing amber mutants in other genes. It had been known that T4 had genes for several tRNAs and he was able to show in one su$^+$ strain that a serine tRNA has the appropriate alteration in its anticodon.

C. Transfer RNA

It is clear that transfer RNA molecules are well-defined three-dimensional structures, although each may perhaps have more than one configuration. It is important to discover these structures, not only to find how each transfer RNA is recognised by its activating enzyme but also to obtain some insight into how the transfer RNA works on the ribosome.

Several stereochemical models for tRNA have been proposed including

a rather attractive and well-defined one by Michael Levitt[f] of the Structural Studies Division. We have approached the problem both by the direct method of X-ray crystallography and by indirect methods which may allow a choice between the different models (138). The latter work has been done mainly by Dr. John Smith and his collaborators.

One indirect method is the chemical modification of bases in tRNA, particularly by selective reagents such as methoxymine. Dr. Tony Cashmore (122, 149) studied the availability of bases to this reagent both in normal transfer RNAs and in the single base mutants of the tyrosine transfer RNA. In some of these mutants additional bases become exposed to chemical attack, and Cashmore was able to show that there is normally an interaction between the dihydrouracil loop and the variable loop region, a result consistent with Levitt's model[f].

Mutant tRNA molecules can be used to study how the synthetase and various ribosomal components recognize tRNA specifically. Drs. Malcolm Gefter and Dick Russell (112) showed that in the tyrosine tRNA the isopentenyl group (normally on the adenine residue adjacent to the anticodon) is essential for codon recognition. This modification is presumably important in the anticodon loop structure.

Several base substitutions specifically affect different tRNA functions but no specific recognition sites have yet been identified. We have isolated several double mutants in which one base change compensates for the functional defects produced by another (103, 120, 139). These show that the exact identity of the base pairs at some positions of the tRNA clover-leaf structure are unimportant for function.

We are presently studying a special class of mutants whose properties

suggest they accept an incorrect amino acid. It is hoped these may lead to the identification of the part of the sequence recognised by the synthetase.

It is fair to say that while this work has not yet converged on a generally acceptable model, many results have been obtained which the final model will have to explain. As in the parallel case of protein structure, the most revealing method would be to solve the crystal structure by X-ray diffraction. It is thus not surprising that in many laboratories a large effort has been made to do just this. We were practically the first laboratory to obtain crystals of tRNA (86). Quite unexpectedly, it has proved rather easy to obtain such crystals and many different crystal forms have been grown and their unit cells characterised. What has proved baffingly difficult is to obtain crystals with sufficient resolution for structure determination. Moreover, even with suitable crystals it will still be necessary to obtain isomorphous replacements with heavy atoms. So far no laboratory has succeeded in this.

Our work has been organised by Dr. Brian Clark with the help of Drs. Klug and Finch (in the Division of Structural Studies) on the X-ray side. A different kind of tRNA is now being tried and it is hoped that this will eventually lead to acceptable crystals and a solution of the structure. We are also attempting, in collaboration with Dr. Brian Hartley's group, to obtain crystals of a tRNA molecule bound to its activating enzyme.

An unexpected dividend of the work on tRNA mutants was the discovery by Dr. Sidney Altman (142) of a precursor for the tyrosine tRNA of E. coli. He and Dr. John Smith (144) have recently sequenced this interesting molecule. It has extra bases both before and after the actual tRNA sequence. The 5' segment is 41 residues long and since it begins with the triphosphate residue,

pppG, it must be the intact transcription product. The enzymatic processing of the precursor is under active study and a specific endonuclease which removes the 5' segment has been isolated. Since many of the mutant tRNAs are defective in their processing, the role in this step of the conformation of the precursor is particularly interesting.

Dr. Arturo Yudelevich studied a leucine transfer RNA of E. coli which is specifically cleaved after phage T4 infection (128, 169).

3. CELL BIOLOGY

A. Nematodes

As has been explained, in the mid-1960's our interest began to turn to problems of higher organisms, particularly those concerning development and the nervous system. The latter is by far the most elaborate structure developed in an organism. Its function depends not only on the performance of elementary biochemical functions (such as the synthesis, release and recognition of neurotransmitter substances and the propagation of a nerve impulse) but also on the exact way the neurons are connected to each other and to effector cells. Many invertebrates, unlike the higher vertebrates, appear to have rigidly defined wiring diagrams, apparently similar in different individuals of the same species, suggesting that the production of the connections is under strict genetic control. We recognised the need for a suitable experimental organism on which both genetical and anatomical studies could be rapidly carried out and which could later be studied biochemically. The requirements are rather stringent: a small organism, easy to grow in the laboratory with a rapid life cycle and with the appropriate sexual system. Free-living nematodes appeared to meet some of these criteria and, after a survey, the strain Caenorhabditis elegans was chosen.

This work differs from most of the work considered in this report in that it has involved a large investment in the development of methods, but as will be seen, most of the technical problems have now been overcome. The different lines of this work are briefly discussed below.

(i) Genetics

The organism is very suitable for genetical analysis, being a self-fertilizing

hermaphrodite from which recessive mutants can easily be isolated. Rare males are found which can be maintained and used to transfer markers from one strain to another for complementation studies and to map mutants. The animal grows on the surface of agar plates, feeding on bacteria. Its life cycle is rapid, taking $3\frac{1}{2}$ days at 20^{o}C. It can also be stored in liquid nitrogen. Over 800 mutants have been isolated, mainly induced by EMS and 400 of these have been studied. Most of the mutants isolated are morphological or affect the movement of the animal. About 100 genes have been characterized, mapping into six linkage groups and many of the genes have repeated occurrences of mutations. A paper on this part of the work is in preparation. Further genetic work, with the help of Dr. Babu, is continuing.

(ii) Behaviour

Methods are being developed by Dr. Roger Freedman to record the behaviour of the wild type and to compare the mutants with the wild type and each other. Dr. Samuel Ward has developed methods of analysing the sensory response of the animals with a view to isolating mutants defective in response to chemicals.

(iii) Anatomy

By far the greatest effort has gone into developing methods for obtaining and studying serial section electron micrographs. The animal is too small for light microscope studies, and the detail revealed by the electron microscope is essential. For example, synaptic junctions are clearly visible and from the position of the synaptic vesicles one can immediately tell which cell is influencing which at a junction. Since adults are only 1 mm. long and larvae are even smaller it is feasible to obtain complete structures of at least a large

fraction of the nervous system from a few thousand serial sections. However, processing and retrieving this information by hand is an extremely difficult and laborious task. For this reason, a considerable amount of work has been done by White and Brenner on developing a system for doing this by a computer. An extensive set of system programmes has already been written for the Modular 1 computer provided by the Council for this work. By the end of this year inputting of data should be under way.

In the meantime, parts of the nervous system of the wild type and of some mutants have been analysed by hand. The structure of the sensory processes in the head can be worked out from a series of approximately 100 electron micrographs. There are 50 such sensory neurons in the hermaphrodite and 54 in the male, and they terminate in cilia or modified cilia. The number of cells, their positions and types of ending have been found to be identical in more than twenty individuals of the wild type. Several mutants have been found to change this pattern. In one class, the variable notched head mutants, the numbers of processes are the same but their positions are altered; in another mutant the number of cells was changed and some processes were found to be absent and others reduplicated.

More extensive work has been on the retrovesicular ganglion. This is a local grouping of twenty neurons found at the anterior end of the ventral cord. Its structure can be determined from a series of 350 to 500 serial sections. Four wild types have been studied, two hermaphrodites and two males. The positions of the cell bodies, the topology of their processes and the synaptic connections between the cells are the same in all four cases. A total of 16

sets of data from 8 different mutants have been analysed. In some cases, more than one individual of the mutant has been studied. The results are too complex to be described here, but, in brief, we have found extensive changes in the pattern in 6 of these mutants. In some cases the alteration is very similar in different individuals of the same mutant and in different mutants of the same gene; in others, this invariance is not maintained. Because the data are incomplete we cannot yet say how the defect in the nervous system might produce the observed behavioural defects but we think that the complete structures, when they are obtained, will be most revealing.

(iv) Embryology

The alterations to the nervous system which we see in the mutants must have their origin in the developing embryo inside the egg. It is thus essential to study the anatomy and especially the neuroanatomy of the developing egg. The tough chitin shells of the eggs give rise to formidable technical problems, but we have mounted an initial attack on this and more work will be done on this problem this year.

(v) Biochemistry

Methods have been developed for growing the animals axenically and for mass cultivation. It is easy to produce $\frac{1}{2}$Kg of stock, all genetically homogeneous, on a laboratory scale. Dr. John Sulston has begun to characterize its DNA. Using an annealing procedure, he has shown that the genome of Caenorhabditis elegans contains some repetitious DNA. The unique sequence length corresponds to 18 times that of Escherichia coli. Some of the implications of this result will be considered later.

B. Positional information

In the development of a higher organism cells must often decide on their

position within a tissue. For example, the work of Sperry, Gaze and others had shown that the ganglion cells of the vertebrate retina know their appropriate position, since their axons can often grow to the correct place in the animal's brain. Wolpert[g] has introduced the term positional information to describe the general phenomenon.

We decided that a 2-dimensional system would be easier to work on than a 3-dimensional one. Wigglesworth[h] had argued eloquently for the advantage of the insect epidermis for such studies. It consists of only a single layer of cells, all of which can show polarity, and moreover it is fairly accessible to experiment. Locke[i] had already done interesting pioneer work on the wrinkles of the cuticle of Rhodnius. This showed convincingly that a "gradient" of some sort exists in the anterior-posterior direction.

The recruitment of Dr. Peter Lawrence (134) has made possible a more detailed study of this system. The wrinkles, which normally run parallel to each other across the cuticle, perpendicular to the anterior-posterior direction, appear only on the adult and not on the larval stages. The basic experimental technique is to cut out a square of epidermis and replace it rotated through either 90^{o} or 180^{o}. This operation is usually done at the last larval stage.

The altered pattern of wrinkles produced is then examined on the cuticle laid down by the adult. By the use of carefully controlled doses of hormones, an adult can sometimes be induced to moult a second time, thus giving the opportunity for a second look at the pattern after an interval of time.

It is difficult to describe the results in a short space. Briefly, the patterns

produced by the operations are neither the unaltered parallel wrinkles nor those expected if the pattern were merely moved unaltered to the new orientation. For example, a 90^{o} rotation produces an S-shaped curve, the direction of the S depending on whether the 90^{o} rotation was clockwise or anti-clockwise (157).

Our theoretical models mostly involve the conception of a diffusing chemical of some sort (a morphogen) since rather simple calculations have shown (124, 162) that there is at least no easy objection to such a theory. Moreover, the calculations predict, rather surprisingly, that patterns when first set up will necessarily be on a small scale, which appears to agree with the experimental facts, both for plants and animals. However, other kinds of theories (such as pumping theories or signalling theories) are also under consideration (150).

Our basic approach is to assume that the anterior-posterior "gradient" imposes a polarity on each cell in the epidermis. This polarity is then postulated to make the wrinkles run perpendicular to the polarity when the adult cuticle is laid down after the moult. Thus the wrinkles roughly follow the contours of the postulated gradient.

Mrs. Mary Munro has tested various mathematical models on a computer by comparing the calculated contour patterns with the broad features of the experimental wrinkle patterns. The results make it unlikely that a simple model based on the pure diffusion of some chemical can be correct. A much better fit is given by models which postulate that each cell has a homeostatic mechanism which attempts to bring the local concentration of the morphogen to a preset level originally fixed by the gradient. The

results suggest that the level for a particular cell is reset whenever that cell divides.

Dr. Lawrence has also done other types of operation on Rhodnius and a certain number on the milk-weed bug Oncopeltus. It is hoped to produce a detailed paper on all this work in the coming year.

Since two dimensions are better than three we argued that one dimension might be superior to two. For this reason, we made the rather unorthodox choice of a prokaryote, the blue-green alga Anabaena.

This forms long chains of cells. The cells are mainly of one type, but there is a minority of cells called "heterocysts" which occur at intervals in the chain and probably fix nitrogen. The latter type never divide. The former always do. Occasionally, a vegetative cell turns into a heterocyst, so that the fairly regular spacing of heterocysts is preserved as the chains lengthen (141).

One advantage of this system, which is being studied by Drs. Michael Wilcox and Graeme Mitchison, is that one can observe the cells constantly and record them by time-lapse photography. In particular, one can see them in the process of division, which is not possible (for technical reasons) in the cuticle of Rhodnius. The work has so far established that, at least in Anabaena cylindrica, a pro-heterocyst will not start to form unless it has a group of a sufficient number of vegetative cells to support it. Moreover, if this support is not maintained adequately (because of competition from other pro-heterocysts or because vegetative cells are artificially removed) the pro-heterocyst may regress to a vegetative cell. A mathematical model is being developed to account for this behaviour.

C. Membranes

Membranes can be approached from many points of view. One could study their lipids, the activity of the proteins they contain (which are often there in only small amounts) or the structure of the proteins present in larger amounts. Dr. Mark Bretscher has chosen to do the latter, starting with that classical object of research, the red blood cell (145, 146, 147). He has developed a chemical reagent, which is highly radioactive, will not penetrate intact cells to any appreciable extent and which attaches itself chemically to certain groups on proteins. By comparing the labelling of intact red blood cells and of their "ghosts" and by an ingenious use of acrylamide gels and finger-printing techniques, he has shown that two of the major components of the red cell surface (one of which is the MN blood group substance - a glycoprotein) are in the membrane, in the sense that one part of each of them is located on the outer surface and another part on the inner surface of the membrane.

This work, which is only beginning, is compatible with other recent work elsewhere which suggests that the protein components of membranes are embedded in a sea of lipid - the bimolecular layer, mainly of phospholipid - in which at least some of them can diffuse slowly from place to place. Bretscher is now actively extending his method to other types of cell.

In parallel with the work described above, an American visitor, Dr. Jay Brown, started to study the peptide-polysaccharides released from cell surfaces by digestion of intact cells with trypsin (148). The preliminary results show that fractionation of these released glycopeptides on DEAE-cellulose columns gave, for the charged material, only four peaks, the amounts

of which vary characteristically from one type of cell to another and even, in one case, during the mitotic cycle. The exact implications of these results are not clear since we do not know how homogeneous each peak is, but an optimistic view would be that the common polysaccharides on cell surfaces may not have as great a complexity as one might have feared.

Since Dr. Jay Brown has now returned to the States we have not yet decided whether to pursue this approach further. What is clear is that there is a great need for methods to characterise polysaccharide sequences rapidly using small amounts of material. The subject still seems to be waiting for its Sanger.

D. Fibrillar elements

During the course of exploratory work on neurons in tissue cultures, derived from chick embryos Drs. Dennis Bray and Dick Fine observed that 40% of the soluble proteins of these cells consisted of two proteins present in approximately equal amounts, one of molecular weight 55,000 and the other of 45,000. The former was shown to be tubulin, the protein of microtubules. More recent work (152) has shown that tubulin consists of two similar but not identical proteins, a result obtained simultaneously in other laboratories.

The other component was more of a surprise. Its length and diameter (as seen in the electron microscope) suggested it might be similar to actin. This was confirmed by a detailed study of its "fingerprint". In fact, so similar is it to muscle actin that to date it has not been possible to establish any difference between them, though small differences could exist (153).

It had previously been shown by others that an actin and a myosin occur for example in the acellular slime mould Physarum and that certain nerve cells contain a protein which reacts with heavy meromyosin as if it were an actin (the test being the appearance of the complex in electron micrographs) but the large amount and the very close similarity to muscle actin came as a surprise. We have also shown that cytoplasmic actin occurs in other tissues of the chick, for example, in lens, lung and skin. A protein of the same size as myosin can be seen in the acrylamide gels but it has not yet been carefully characterised.

We intend to pursue this work because of its intrinsic importance. It seems highly likely that the shape of cells, all movement within cells and all movements of cells as a whole are controlled by fibrillar elements. In animals these are likely to be proteins. The first stage is to discover how many families of fibrillar elements exist. Those already known are tubulin, actin and myosin. A possible fourth class, the so-called neurofilaments, is so far inadequately characterised. It will then be necessary to identify how many sub-classes there are in each family, and to find how they are distributed in Nature. In particular, do plants have actin and myosin? For all these problems the techniques are already available.

The next step will be to discover how they interact to produce movement and shape. For example, do neurofilaments slide on microtubules? To do this it will probably be necessary to study their behaviour in the test-tube or in glycerinated cells (as has been done for muscle proteins). This is certainly feasible but probably more difficult than the earlier steps.

The hardest problem will be to find out how the fibrillar elements are

persuaded to occupy their correct positions in a cell and how their activities are controlled. As yet, no good ideas exist about how this might be done.

E. Chromosome structure

Over the past year or so we have become increasingly interested in the genetic material of higher organisms, and recently Crick has formulated a model of the chromosome which unifies many different experimental results (151).

One consequence of this model is worthwhile emphasizing. It states that most of the DNA in higher organisms is used for control purposes and only a very small fraction codes for polypeptide chains. This means that the number of different proteins to be found in higher organisms is probably of the order of a few thousands or tens of thousands and that their elaborate structures must stem from a complexity of control elements rather than from millions of new elementary functions.

The genetic and biochemical results we have obtained with Caenorhabditis are consistent with this theory which explains rather well the paucity of complementation groups in this and other organisms. We know that some single gene mutations can produce profound changes in the organisation of the nervous system of the nematode but we do not know whether these mutations are due to alterations of control elements in the DNA or due to alterations or absences of proteins. We have already begun to pursue this problem in the nematode by screening other mutagens and exploring other kinds of mutants. Even so we are not sure if we can bring this kind of work to the molecular level. Genetical studies may only reveal to us something of the broad structure of the gene and the logic of the genetic

programme rather than the detailed molecular mechanisms involved. For this reason, we are also starting several lines of biochemical work to try to prove or disprove the cardinal features of the model.

One of these is the exploration of the mechanism of transcription in higher organisms. Dr. Andrew Travers had continued the work he began at Harvard on bacterial transcription factors but has also begun to study RNA polymerase in eukaryotes. For technical reasons, yeast was initially chosen for this work and he has purified some of the polymerases from this organism. However, in the light of the new ideas, it is not certain whether this is the best choice of a eukaryote and it is likely that the work will be extended to other organisms next year.

In addition, we have been considering, with Dr. Aaron Klug, the structure of chromatin as revealed by X-ray diffraction and other methods. This has already led to a tentative model and experimental work is now being planned.

4. MISCELLANEOUS

A. Theoretical work.

The main effort of our laboratory has been experimental but theoretical papers have inevitably arisen as well. Here we list them only briefly. Jacob and Brenner put forward the concept of the replicon (17, 18). Crick and Orgel discussed inter-allelic complementation (25). Crick proposed the "wobble" theory for the degeneracy in the recognition of the third base in the codon (55). Brenner and Milstein suggested a form of hypermutation as a possible mechanism to produce antibody variation (51). Crick wrote on the origin of the genetic code (91), this being a companion paper to one by Orgel[j] on the origin of life. Bretscher (80) proposed a model for translocation in protein synthesis. Crick (125) was forced to defend the so-called Central Dogma, originally put forward in 1958, but confused by Watson in his text book. More recently Crick has proposed a general model for the chromosomes of higher organisms (151). This has already been mentioned in this report.

In addition, numerous review articles or articles of a general nature were written during this period by members of the Division.

B. Other lines of work in progress.

The Division is also engaged in other lines of work but these are in progress and have not reached the stage where we wish to discuss them. These include the following:-

(1) The mechanism of secretion of proteins, at present mainly in prokaryotes (Dr. Mary Osborn)

(2) Myoblast fusion (Dr. Clark Slater)

(3) Cell surface proteins (Dr. Hannah Friedman)

(4) Tissue culture (Mrs. Leslie Barnett)

(5) Synaptic vesicles from the adrenal medulla (Dr. John Phillips).

In addition, Dr. David Marr has joined us recently to continue his work on the theory of the higher nervous system.

C. Work discontinued.

As part of our venturing into the field of higher organisms various lines of work were begun which have since been discontinued. We mention these briefly below.

1. Work on Ascaris lumbricoides

A project was begun by Dr. A.O.W. Stretton on Ascaris a large parasitic nematode, in which it was thought that neurophysiology might be done to complement the studies on Caenorhabditis elegans. Dr. Stretton had done extensive work on the light microscopic anatomy of Ascaris but whether or not electrical recordings can be made of its neurons is still not known. Dr. Stretton has recently left the laboratory and taken this problem with him. We doubt whether we ourselves will continue this work since at the moment our interests lie more in the developmental and the genetical problems associated with nervous systems rather than in the characterization of their activity.

2. Immunology.

Dr. Alan Munro initiated research on the immunological response of lymphocytes in vitro. This work has led to interesting results (132, 136) but our Division did not have the resources to support it on the scale needed and it was not connected closely enough with our other interests. Dr. Munro

now has an appointment in the Cambridge University Department of Pathology where he is continuing this work.

5. FUTURE WORK

As can be seen from the main body of this report, the more recent work of the Division is very varied in character. The effort on the small nematode is a major one. It is well established and so far no other laboratory has taken up the problem on this scale. As a genetic system it is rivalled only by Drosophila. It is likely to be widely adopted for genetic purposes when Brenner's paper is published.

The neuroanatomy on the other hand though clearly possible is unlikely to be imitated by many laboratories because of the scale of effort needed and the investment involved in both time and money. The embryology is of unknown difficulty and it is a matter of high priority to find out just how difficult it is. In addition, we intend to use the nematode for problems in other fields, such as obtaining mutants of muscle proteins and, hopefully, distinguishing between mutants which alter proteins and those which disturb only regulation.

The work on positional information is likewise not, at the moment, being pursued elsewhere on a big scale. The number of people in the world interested in the problem is relatively small and at least half of them are in England. It will take several more years of research before we have a good measure of the problem. The crucial step will be to go from the stage of making mathematical models to that of biochemistry. Just how difficult this will be remains to be seen. When it happens the field is likely to attract a flood of new recruits.

By contrast, the fields of membranes and fibrillar proteins are already fairly well populated and out contributions do not yet hold the centre of the stage. Yet both fields are of central importance to developmental

biology and cannot fail to interact eventually with the work on nematodes and on positional information. We seem to have made a useful start in both of them and can see plenty of immediate problems to tackle.

The field of chromosome structure is chaotic. The experimental work ranges over many separate areas - histones, genetic mapping and complementation, heterogeneous nuclear RNA to name only a few - and involves many different techniques, from nucleic acid hybridisation to the X-ray diffraction of chromatin. Much of this work is of doubtful quality. We have not yet finally decided which areas to concentrate on, but we are already promoting collaborative work in some of them. The problem itself is of the first importance, especially the nature of the control mechanisms at different levels, and is central to everything else in developmental biology. Whether the new model will provoke a breakthrough remains to be seen but it has already served to sharpen the experimental approaches being used.

Some of the other lines of work we have mentioned briefly we regard as tentative. They may be shut down or expanded depending on the way they develop. In any case, we should like to reserve some space and effort for new approaches which may appeal to us as we get deeper into our subject.

Finally, we should mention Dr. John Gurdon and his group who will be joining us in the summer. They have an enviable reputation for experimental work which is both bold, significant and critical. His work, using Xenopus oocytes or eggs as living test-tubes appeals to us immensely, and we look forward to an exciting period of active collaboration between these new members of the Division and the present ones.